20. (New) The method of claim 8, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 20-38.

B9 Cont

21. (New) The method of claim 8, wherein said polypeptide, when combined with an RNA subunit to form an RNase P holoenzyme, has at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme.

REMARKS

The present invention provides RNase P polypeptides and methods for identifying antibiotics using these polypeptides.

Claims 1, 2, 8-11, 13, and 14 were examined in this case. Claims 2, 9, and 13 were rejected under 35 U.S.C. § 112, second paragraph, and claims 8-11, 13, and 14 were rejected under 35 U.S.C. § 112, first paragraph. Claim 1 was rejected under 35 U.S.C. § 102, and claims 8,10, 11, 13, and 14 were rejected under 35 U.S.C. § 103. Claims 2 and 9 were determined to be free of the prior art of record. Each of these rejections is addressed below in the order that they appear in the Office Action.

Support for the Amendments

The paragraph on page 22, lines 10-21 of the specification has been amended to indicate that the reaction buffer can contain 1-10 mM DTT, as indicated in original claim 14.

Claim 1 had been amended to exclude the bacterial amino acids sequences in the RNase P database (James W. Brown, The Ribonuclease P Database, Nucleic Acids Research 27(1):314 (1999)) posted on the internet on March 1, 2000 and to exclude the *Staphylococcus aureus* and *Streptococcus pneumoniae* amino acid sequences described in WO 99/11653 and EPO 811 688. As stated on page 7, lines 17-22, of the specification, these polypeptides are specifically excluded from the polypeptides of the present

invention. In particular, the attached Declaration of patent agent Dr. Vicki Healy lists the bacterial and eukayrotic RNase P protein sequences that James Brown, the founder of the Ribonuclease P Database, indicated were available in his RNase P Database (RNase P) on March 1, 2000. Amended claim 1 and new claim 19 specify that the polypeptide is a bacterial polypeptide (see, for example, page 1, lines 8-10).

Claim 9 was amended to include the definition of "substantially identical" (page 7, lines 1-16 and 23-25), and claim 13 was amended to clarify the antecedent basis of a phrase in the claim and to recite 10-40 μg/ml carbonic anhydrase (see, for example, page 22, lines 18-19). New claims 15 and 20 specify that the polypeptides have an amino acid sequence with 100% identity to particular amino acids in the RNase P consensus sequence (see, for example, page 7) and new claims 16 and 20 specify that the polypeptides that have an amino acid sequence at least 95% or 100% identical to any one of SEQ ID NOS: 20-38 (as disclosed, for example, on page 6). New claims 17 and 21 require that the polypeptides referred to in the claims, when combined with an RNA subunit to form an RNase P holoenzyme, have at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme (see, for example, page 4). New claim 18 specifies that the buffer has 2-10 mM DTT (see, for example, page 22, lines 20-21).

Applicants note that new claims 16 and 20 recite the elected species SEQ ID NO: 27 (*Neisseria gonorrhoea*).

In view of the election of claims 1, 2, 8-11, 13, and 14, claims 3-7 and 12 have been canceled.

These amendments add no new matter. A marked-up version indicating the amendments made to the specification and claims, as required by 37 C.F.R. § 1.121(b)(1)(iii) and (c)(1)(ii), is enclosed.

Objection to the Specification

The Examiner states that the specification is objected for failure to provide unique sequence identification numbers for each amino acid sequence within the specification. In response to this objection, the specification has been amended to include SEQ ID NOs:39-95, which refer to their corresponding amino acid sequences.

As required by 37 CFR 1.825(a), enclosed is an amended sequence listing consisting of 31 sheets to be inserted at the end of the application. The sequence listing has been amended to include SEQ ID NOs: 39-95 from Fig. 1 and contains no new matter.

As required by 37 CFR 1.825(b), also enclosed is a diskette containing a copy of the sequence listing in computer readable form, including all previously submitted data with the amendments incorporated therein. The contents of the computer readable form are the same as the contents of the paper sheets. Accordingly this objection may now be withdrawn.

The Examiner also states that the specification is objected for referring to Fig. 2 as a figure containing a three-dimensional structure. Applicants have eliminated this reference to Fig. 2. Accordingly, this rejection can also be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 2 and 9 were rejected under 35 U.S.C. § 112, second paragraph, for reciting non-elected embodiments of the invention. As indicated on page 2 of Office Action mailed August 29, 2001, the single species SEQ ID NO: 27 (*Neisseria gonorrhoea*) is elected for prosecution on the merits. In the event that no generic claim is allowed, the claims shall be restricted to the elected species. Thus, applicants respectfully assert that claims 2 and 9 need not be limited to the elected species at this time. Such an amendment would be potentially appropriate when no generic claim is allowed. In the event that a generic claim is allowed, applicants assert that claims to the remaining

species, which are written in dependent form, or which otherwise include all the limitations of the allowed generic claim should be considered as provided by 37 CFR § 1.141 and MPEP § 809.02(a). Accordingly, this rejection should be withdrawn.

Claim 9 was also rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite for use of the phrase "substantially identical." In view of this rejection, applicants have amended claim 9 to include the definition of "substantially identical" found on page 7, lines 1-16 and 23-25, of the specification. This rejection may now be withdrawn.

Claim 13 was rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite for lack of antecedent basis for the phrase "said fluorescence analysis." Applicants have amended claim 13 to eliminate this phrase and to replace it with "said contacting," which has antecedent basis in step (ii) of claim 8. This rejection may now be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claim 14 was rejected, under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner states that claim 14 refers to a buffer containing 1-10 mM DTT, while the specification refers to a buffer containing 2-10 mM DTT. The specification has been amended to also refer to a buffer containing 1-10 mM DTT (as disclosed in original claim 14). Accordingly, this rejection can now be withdrawn.

Claim 8-11, 13, and 14 were rejected, under 35 U.S.C. § 112, first paragraph, for lack of enablement for failure of the specification to indicate that antibiotics can inhibit or interfere with RNase P activity. Applicants respectfully assert that numerous sections of the specification indicate that antibiotics can inhibit RNase P activity.

For example, page 1, lines 8-10, of the specification states that

> [t]his invention relates to novel bacterial ribonuclease P protein subunits and their use as *targets* in screening assays to identify compounds useful as antibacterial agents. (emphasis added)

Similarly, page 2, lines 9-12, of the specification states that

> [m]any pathogens exist for which there are few effective treatments and the number of strains resistant to available drugs is continually increasing. Accordingly, novel compositions and methods for assaying RNase P function would be useful for identifying antimicrobial compounds against these pathogens.

And page 2, lines 19-22, states that

> The invention also features methods of using these sequences identify additional RNase P nucleic acids and proteins, and methods to screen for compounds which *inhibit the RNase P function.* Such compounds can be used as *antibacterial agents.* (emphasis added)

Based on the above teachings that the RNaseP polypeptides of the invention are useful **targets** in assays for the identification of **antibacterial** agents, one skilled in the art would appreciate that the identified antibiotics inhibit RNase P activity. Moreover, the specifications describes assays such as the following in which antibiotics are selected based on their inhibition of RNaseP holoenzymes:

> In the fifth aspect, the invention features a method of identifying an antibiotic agent, said method including: i) obtaining an RNase P holoenzyme comprising the polypeptide of the first aspect of the invention; ii) contacting the holoenzyme with an RNase P substrate in the presence and in the absence of a compound; and iii) measuring the enzymatic activity of the holoenzyme; wherein a compound is identified *as an antibiotic agent if said compound produces a detectable decrease in said RNase P enzymatic activity* as compared to activity in the absence of the compound. (page 3, lines 11-18, emphasis added)

Given the teachings in Applicants' specification of the usefulness of compounds that inhibit the RNase P polypeptides and holoenzymes of the invention as antibiotics and given the important role of RNase P in the cleavage of 5' terminal leader sequences of precursor tRNAs in bacteria that was known at the time of filing (see, for example, page

1, lines 11-13), a skilled artisan would realize that compounds that inhibit RNase P activity are useful as antibiotics. In view of these clarifying remarks, this rejection should be withdrawn.

Rejection under 35 U.S.C. § 102

Claim 1 was rejected, under 35 U.S.C. § 102(b), as being anticipated by Gress (WO 99/11653), Guth (EP 0 811 688), Altman (The RNA World, 2:1155-1184, 1999, and FASEB Journal, 7:7-14, 1993), Frank (Annu. Rev. Biochem., 67:153-180, 1998), Gopalan (J. Mol. Biol., 267:818-829, 1997), Pace (J. Bacteriol., 177:1919-1928, 1995), Pascual (Proc. Natl. Acad. Sci. USA, 96:6672-6677, 1999), or Peck-Miller (J. Mol. Biol., 221:1-5, 1991), with the Examiner stating that each of these references teaches a consensus sequence for RNase P.

These above-cited references focus on the following bacterial RNase P subunits or complexes *S. pneumoniae* (Gress), *Staphylococcus aureus* (Guth), *E. coli* (Altman, 1999), *E. coli*, *Baccillus subtilis*, *Proteus mirabilis*, *Streptomyces bikiniensis*, and *Micrococcus luteus* (Altman, 1993), *E. coli*, *Baccillus subtilis* (Frank), *E. coli, Buchnera aphidocola, Coxiella burnstii, Haemophilus influenzae, Proteus mirabilis, Pseudomonas putida, Mycoplasma capricolum, Mycobacterium leprae, Micrococcus luteus, Streptomyces coelicolor*, and *Bacillus subtilis* (Gopalan), *E. coli* and *Baccillus subtilis* (Pace), *E. coli* and *Synechocystis* (Pascual), and *E. coli* (Peck-Miller). As noted above, these RNase P polypeptides have been excluded from amended claim 1. As none of these references disclose RNase P polypeptides that fall within the scope of amended claim 1, these 102 rejections may now be withdrawn.

Rejection under 35 U.S.C. § 103(a)

Claims 8, 10, 11, 13, and 14 were rejected, under 35 U.S.C. § 103(a), as being

unpatentable over Patuschak (Nucl. Acids Res. 21:3229-3243, 1993), Mikkelsen (Proc. Natl. Acad. Sci., USA, 96:6155-6160, 1999), or Schroeder (EMBO J., 19(1):1-9, 2000). None of these references, either alone or in combination, disclose or suggest the RNase P polypeptides recited in the present claims or their use in screening assays to identify antibiotics. Thus these references do not render claim 8, 10, 11, 13, or 14 obvious. This rejection should be withdrawn.

CONCLUSION

In summary, applicants submit that the claims are now in condition for allowance, and such action is respectfully requested. Also enclosed are a petition to extend the period for replying for three months, to and including October 11, 2002 and a check for $460.00 for the required fee. Also enclosed is a check for $9.00 for the excess claim fee. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: October 11, 2002

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I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

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Elaine Fabrizio
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Venkat Gopalan et al.	Art Unit:	1652
Serial No.:	09/516,061	Examiner:	Charles L. Patterson Jr.
Filed:	March 1, 2000	Customer No.:	21559
Title:	NOVEL BACTERIAL RNASE P PROTEINS AND THEIR USE IN IDENTIFYING ANTIBACTERIAL COMPOUNDS		

Commissioner For Patents
Washington, D.C. 20231

Version with Markings to Show Changes Made

In the specification:

A marked-up version of the paragraph on page 8, lines 22-27, of the specification is presented below.

> Fig. 1 shows the sequence alignment of previously known bacterial RNase P protein subunits using the ClustalW alignment program (Thompson et al., Nucleic Acids Research 22: 4673, 1994) and the alignment of the RNase P sequences of the present invention (SEQ ID NOs: 39-95). [The aligned fragments of the known RNase P sequences are designated by (*) and the aligned fragments of the RNase P sequences of the invention are designated by (#).]

A marked-up version of the paragraph on page 10, line 27 through page 11, line 8 of the specification is presented below.

This RNase P consensus sequence was derived as follows. We aligned the sequences of the known bacterial RNase P protein subunits using the ClustalW alignment program (Thompson et al., *supra*) (see Fig. 1, the previously known RNase P sequences [are designated by (*) and] were obtained from the RNase P database; www.jwbrown.mbio.ncsu.edu/rnasp/home.html.) This ClustalW alignment was then manually refined to align highly conserved RNase P hydrophobic and basic residues that had been demonstrated by mutation studies to be important for RNase P catalytic function (Gopalan et al., J. Mol. Biol. 267: 818, 1997). The spacing between the conserved residues, as well as the identity of the individual residues, appears critical to RNase P function.

A marked-up version of the paragraph on page 11, lines 9-25, of the specification is presented below.

Twenty amino acids were identified as highly conserved (shown as the shaded residues in Fig. 1). The percent of RNase P sequences which conserve each of the shaded residues is shown below the sequence information as percent identity. Based upon these known sequences, we determined that a polypeptide identified by our above-described RNase P BLAST search contained an RNase consensus sequence and was a genuine RNase P protein subunit if it contained at least nine of the above-described twenty amino acids. Preferred polypeptides have a consensus sequence with at least 13 of the amino acids and/or conserve at least seven of the following subset of amino acids: F18, R46, K53, A59, R62, N63, K66, R67, and R70. This subset of amino acids is preferred because it has been identified as playing a significant role in RNase P function through mutation studies (Gopalan et al., J. Mol. Biol. 267: 818 1997) and the determination of the RNase P three dimensional structure (Stams et al., Science 280: 752, 1998). [As shown in Fig. 2, the] <u>The</u> three dimensional structure reveals that all of the residues that make up the above-described nine amino acid subset are proximal to each other in the tertiary structure of the protein, despite the distance between some of the residues in the primary structure.

A marked-up version of the paragraph on page 12, lines 9-14, of the specification is presented below.

All of the novel RNase P protein sequences were identified by the above-described BLAST search. The alignment of these sequences with the known RNase P sequences is also shown in Fig. 1 [(the RNase P sequences of the present invention are designated by (#))]. This alignment demonstrates that the amino acid sequences of the invention all contain RNase P consensus sequences. Therefore, these polypeptides are genuine RNase P proteins.

A marked-up version of the paragraph on page 22, lines 10-21, of the specification is presented below.

The preferred reaction buffer contains 50 mM Tris-HCl (pH 7.5), 100 mM ammonium chloride and 10 mM magnesium chloride. Concentrations of 10-100 mM, 25-500 mM and 1-100 mM of the above, respectively, can be substituted, as can other buffering agents such as MOPS or HEPES, or other monovalent cations, such as sodium or potassium. When the assay is run in either 98 or 384 [364]-well polystyrene or polypropylene assay plates, there is a very significant decrease in the fluorescence intensity and polarization of the annealed substrate over time in the absence of enzyme. Various conditions have been tested to prevent the loss of signal with time. The preferred conditions include addition of 10-40 μg/ml carbonic anhydrase and 10-100 μg/ml polyC to the buffer. Other materials, such as, 0.5-5% glycerol, 10-100 μg/ml hen egg lysozyme, 10-50 μg/mL tRNA, 1-10 mM DTT, or 2-10 mM DTT can also be added to the buffer to prevent some loss of signal.

In the claims:

A marked-up version of claims 1, 9, and 13 and new claims 15-21 are presented below.

1. (Amended) An isolated polypeptide comprising an RNase P consensus sequence wherein said polypeptide has RNase P protein activity, wherein said polypeptide is a bacterial polypeptide, and wherein said polypeptide is not a polypeptide from one of the following organisms: Coxiella burnetii (None Mile) U10529, Rickettsia prowazekii (Madrid E) AJ235272, Neisseria meningitidis (Z2491) AL162753, Neisseria meningitidis (MC58) AE002540, Buchnera aphidocola (unspecified) M80817, Buchnera aphidocola (SGS) AF008210, Buchnera sp. (APS) AP000398, Haemophilus influenza (RD KW20) U32848, Escherichia coli (unspecified) M11056, Escherichia coli (K-12) AE000394,

Proteus mirabilis (unspecified) M58352, Pseudomonas aeruginosa (PAO1) AE004968, Pseudomonas putida (unspecified) P25752, Salmonella typhi (CT18) no accession number, Yersinia pestis (Orientalis) no accession number, Xyelella fastidiosa (unspecified) AE004083, Campylobacter jejuni (NCTC 11168) AL139076, Helicobacter pylori (26695) AE000645, Helicobacter pylori (J99) AE001557, Micrococcus luteus (S66) U64884, Mycobacterium avium (104) AF222789, Mycobacterium bovis (AF2122/97) no accession number, Mycobacterium leprae (Lortist 6) L39923, Mycobacterium tuberculosis (H37Rv) AL021426 X92504, Streptomyces bikiniensis (Zorbonenis) M83112, Streptomyces coelicolor (A3(2)) M82836 AL049826 AF031590, Bacillus halodurans (C-125) AB013492, Bacillus subtilis (168) X62539 AL009126, Mycoplasma capricolum (mcs5) P14982, Mycoplasma genitalium (G-37) U39713, Mycoplasma pneumoniae (M-129) U00089, Staphylococcus aureus (ISP3) AF135268, Ureaplasma urealyticum (3/1) AE002158, Pseudanabaena sp. (PCC6903) J000513, Synechocystis sp. (PCC6803) X81989, Borellia burgdorferi (212) Z12166, Borellia burgdorferi (B31) AE000783, Treponema pallidum (Nichols) P50069, Chlamydia trachomatis (serovar D) AE001351, Chlamydia muridarum (trachomatis MoPn) AE002160, Chlamydophila pneumoniae (CWL 029) AE001673, Chlamydophila pneumoniae (AR39) AE002251, Deinococcus radiodurans (R1) AE002049, Thermotoga maritima (MSB8) AAD36531, B.burgdorferi, B.burgborferi-partial, C.burnetii, C.pneumoniae-2, C.trachomatis, H.influenza, H.pylori-48, M.leprae, M.luteus, M.tuberculosis-2, M.bovis, Pseudanabaena-6903, R.prowazeki, S.bikiniensis, Synechocystis6803, Staphylococcus aureus, and S. pneumoniae.

9. (Amended) The method of claim 8, wherein said polypeptide [is substantially identical to a polypeptide of SEQ ID NOS:20-38] has at least 95% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

13. (Amended) The method of claim 11, wherein said contacting [fluorescence analysis] is carried out in a buffer comprising 10-40 μg/ml [mg/ml] carbonic anhydrase and 10-100 μg/ml polyC.

15. (New) The polypeptide of claim 1, having 100% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

16. (New) The polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOS: 20-38.

17. (New) The polypeptide of claim 1, wherein said polypeptide, when combined with an RNA subunit to form an RNase P holoenzyme, has at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme.

18. (New) The method of claim 14, wherein said buffer comprises 2-10 mM DTT.

19. (New) The method of claim 9, wherein said polypeptide has 100% identity to the corresponding twenty amino acids: R11, L12, F18, R46, G48, V51, K53, K54, A59, V60, R62, N63, K66, R67, R70, L80, D84, V86, L101, and L105 of *E. coli* RNase P.

20. (New) The method of claim 8, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 20-38.

21. (New) The method of claim 8, wherein said polypeptide, when combined with an RNA subunit to form an RNase P holoenzyme, has at least 20% of the enzymatic activity of an *E. coli* or *B. subtilis* RNase P holoenzyme.